Laboratory Diagnosis of Tuberculosis
Page 3

Role of Histopathology Laboratory in the Diagnosis of Medical Renal Disease
Page 11

Application and Uses of Flow Cytometry
Page 23

Role of the Pathologist in the Management of Breast Diseases
Page 37
From the Editor’s Desk

This issue of our Lab Communiqué focuses on the Lab diagnosis of several important medical and surgical problems encountered in routine practice. Namely, Tuberculosis, Renal diseases, Breast diseases and haematological disorders. We also look at the futurescape of Pathology and take you through the world of Digital pathology. The optimists would have us believe that digitalization will transform pathology practice as it did the practice of radiology 15 years ago.....

As we enter the era of Personalised medicine and Evidence-based management in medicine, I hope these topics will give you insights in redefining practices and ‘standards of care’.

We lost this year a very respected colleague, Dr Ajita Mehta, and her team-mate of several years, Dr Camilla Rodrigues, pays tribute to her endearing and everlasting qualities.

Dr Anita S. Bhaduri, M.D.
Surgical Pathologist

Contents

2 Editor’s Desk

3 Laboratory Diagnosis of Tuberculosis
Dr. Camilla Rodrigues

11 Role of the Histopathology Laboratory in the Diagnosis of Medical Renal Disease
Dr. Chitra Madiwale

23 Application And Uses Of Flow Cytometry
Dr. Shanaz Khodaiji

37 Role of the Pathologist in the Management of Breast Diseases
Dr. Anita S. Bhaduri

Important Lab Numbers

Department Of Laboratory Medicine ..............
Location- Jamuna Clinic (Hinduja Clinic)
Stat Lab – Reception……………………………2444 7327
Stat Lab – Biochemistry …………………..2444 7328
Blood Bank……………………………………2444 7308
Donor Room …………………………………2444 7306

Ground Floor
O.P.D. Blood Collection………………………2444 7079

Location - Lalita Girdhar Building (S1 Bldg)
Biochemistry Lab …………………..2444 7935/
……………………………………2444 7931
Hematology Lab………………………………2444 7947
RIA Lab……………………………………2444 7948
Microbiology/Serology Lab …………..2444 7793/
……………………………………2444 7794

Histopathology /
Cytopathology Lab…………………………2444 7797
NAT Lab……………………………………2444 7610/
……………………………………2444 7611

Home Collection Service ………….3981 8181/
……………………………………6766 8181
Hinduja Poison Center …………………2446 4600
Lab Medicine Fax Number …………2444 2318

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Laboratory Diagnosis of Tuberculosis

Tuberculosis remains one of the deadliest diseases today despite the availability of effective preventive measures and chemotherapy.

**Tuberculin Skin test (TST) – Mantoux test**
We have celebrated more than 100 years of the TST (developed in the late 1800’s) and it is based on the fact that M. tuberculosis caused cutaneous reactivity to a concentrated filtrate from heat killed cultures of M. tuberculosis. The immunologic basis of this lies in the fact that infection with M. tuberculosis produces a delayed type hypersensitivity (DTH) to certain antigenic components contained in the extract of the culture filtrates.

Since it was first introduced, the tuberculin test has undergone continual refinement in its formulation, standardization, and dosage, as well as its interpretation and indications for use. New guidelines have replaced universal screening with targeted testing and rigid definition of positivity with individualised criteria. The standard skin test dose for RT-23 (WHO – Statens Serum Institute) is 2 TU and this has been matched for bioequivalence to 5TU of Purified Protein Derivative PPD-S (USA).

**Microscopy**
Given the poor specificity of TST, the importance of smear and culture confirmation cannot be overemphasized. For day to day work, detection of AFB in the sample is enough if the clinical and or radiological features suggest the diagnosis. Smear examination by Ziehl-Neelson (Z/N) stain has the advantage of simplicity, availability and rapidity, but the sensitivity is affected by the skill and experience of the microscopist, the number of specimens examined and the concentration of organisms in the sputum. To detect 1-2 organisms in 300 oil immersion fields the concentration of organisms must be 5000-10,000 per ml. Thus under operational conditions positive smears are found in 50-70% specimens. Early and timely diagnosis of tuberculosis relies heavily on microscopic examination of clinical samples for acid fast bacilli using the Ziehl Neelson (Z-N) stain. Microscopy can detect 60-70% of culture positive samples with a lower limit of detection of $5 \times 10^3$ organisms / ml. Typical acid fast bacilli appear as slightly curved, beaded long or short rods.

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Dr. Camilla Rodrigues Consultant Microbiologist, MD - Microbiology
Email ID: dr_crodrigues@hindujahospital.com Contact No. 24451515 ext 7155
The AFB smear report is of value not only in diagnosis but also helps to grade infectivity.

The scale recommended by RNTCP is as follows:

- 3+ = >10 AFB/ oil immersion field
- 2+ = 1-10 AFB/ oil immersion field
- 1+ = 10 - 99 AFB / 100 oil immersion fields
- Exact = 1-9 bacilli / 100 oil immersion fields

**Fluorochrome stains**

The waxy mycolic acids in the cell walls of mycobacteria have an affinity for the fluorochromes, auramines and rhodamine. These dyes non specifically bind to nearly all mycobacteria.

The mycobacterial cells appear bright yellow or orange against a greenish background. This method can be used to enhance detection of mycobacteria directly in patients specimens.

The advantage of this method is that lower magnification can be used; so wider area is covered enabling rapid screening. However the cost of microscope and reagents are high and a very highly trained technician only can screen the smears. Besides the quick fading of the fluorescent stains creates additional practical problems.

**Culture techniques**

**Conventional tests**

**Solid medium based methods**

Culture of mycobacteria is a much more sensitive test than smear examination and allows for biochemical identification of the species considering enhancing the specificity. Unfortunately the slow doubling time of M. tuberculosis makes culture on egg/agar based solid media slow and time consuming. Agar based media allow detection of colonies in 10-12 days, whereas most commonly used Lowenstein Jensen Medium (L.J.) usually takes 18-24 days. Susceptibility testing can also be performed on LJ but the turn around time is approximately 3 months for culture and susceptibility.

**Automated liquid culture methods**:

1. BACTEC TB 460 is a sensitive, specific and rapid culture method for smear positive respiratory as well as non-respiratory specimens.

   Time for detection of M. tuberculosis complex from smear negative clinical specimens is 13-15 days. In specimens, which are difficult to obtain such as tissue biopsies and body fluids, the use of L.J. media and BACTEC TB 460 may be justified to maximize isolation of mycobacteria. Different studies of the sensitivity of TB-BACTEC in
monitoring mycobacterial growth showed that an inoculum of 200 viable M. tuberculosis bacilli could be detected in 12-13 days while as few as 20 viable bacilli could be detected if one waited for 14-17 days.

2. Mycobacterial Growth Indicator Tube (MGIT) 960 TB: The test employs a new state of the art fluorescent technology that enables positivity as rapidly as 7-10 days. It is based on oxygen quenching with fluorescent dye. MGIT 960 TB is used for isolation & accurate identification of mycobacteria and also for susceptibility testing to first and second line anti tubercular drugs by the modified proportion method.

3. Bac T /ALERT employs a colorimetric sensor and reflected light to monitor the production of CO2 in the culture medium. This is detected by a photodetector based on the sustained increase in the reflectance units of the light emitting diode of the system.

**Phage assays**
The ability of mycobacteriophages to lyse and destroy mycobacteria has been explored for rapid diagnosis of TB. The assay uses specific mycobacteriophages (DNA viruses specific for M. tuberculosis complex) as a reporter to reflect the presence of viable TB bacilli in the clinical specimens within 48 hours.

**Serological tests**
The search for a rapid accurate and cheap serological test for the diagnosis of tuberculosis has become as the search for the holy grail. A large number of antigens have been used in assessing the humoral response in tuberculosis, but the fact that none has emerged victorious underscores the poor sensitivity and specificity of serology in endemic areas as India.

The antigens used so far include Antigen 5 (38 KDa antigen), A60 antigen 45/47 KDa antigen complex, BCG, 19KDa 30 KDa antigen, LipoArabinoMannan (LAM) and many more. In majority of studies specific serologic tests for diagnosing active pulmonary tuberculosis have been addressed. The diagnostic dilemmas exist not so much in pulmonary tuberculosis but in paucibacillary extrapulmonary disease where the performance of serological tests is simply not good enough.

A good serological assay should perform well in specific targeted populations especially childhood tuberculosis, HIV positive and
extrapulmonary TB. Unfortunately, the sensitivity of most serological tests falls with smear negativity – a finding attributed to the lower burden of organisms. In addition, the fact that large number of environmental bacteria have cross reactive antigens and the antigens used so far are not very species specific contrive to confuse the diagnosis.

**Interferon γ assays**

An alternative to the tuberculin test are the Interferon – γ assays in the immunodiagnosis of tuberculosis. These assays are based on the principle that tuberculosis antigen sensitized T cells produce γ interferon when they re encounter mycobacterial antigens. These in vitro assays employ extremely specific antigens of M. tuberculosis as Early Secretory Antigenic Target-6 (ESAT-6) and culture filtrate protein 10 (CFP-10 ) called the RD1 antigens. These antigens are not shared by BCG strains and most Non Tuberculous Mycobacteria.In a recent study have been shown to have little diagnostic utility in countries endemic for tuberculosis with 80% of healthy adults in Mumbai responded to this antigen compared to 0% of a similar cohort in the United Kingdom.

A recent meta analysis has shown the sensitivity of these assays in patients with bacteriologically confirmed active tuberculosis varied between 32-100% and in those latent tuberculosis about 80%. The sensitivity was slightly lower than TST.

Two commercial assays are currently available

- The QuantiFERON GOLD assay with both ESAT 6 and CFP-10 uses whole blood to measure γ interferon response to the above antigens
- T SPOT –TB assay uses peripheral blood mononuclear cells (PBMCs) and detects the number of " spot forming T cells" by use of ELISPOT in response to the above RD1 antigens

**Molecular methods**

Advances in molecular medicine have provided the research and diagnostic laboratory with tools that are revolutionising its function. Molecular methods of diagnosis are exciting new developments that are already in practice in developed countries.

1. **Nucleic acid probes**

Nucleic acid hybridization is a powerful and widely used technique which exploits the ability of complementary sequence in single-strand (ss) DNA or RNA to pair with each other to form a duplex. The ss nucleic acid probes used for hybridization should be complimentary to the amplified
sequence or the region of interest on the gene to be identified.

2. In situ hybridization
In situ hybridization is used to detect and locate specific DNA or RNA sequence(s) in tissues or chromosomes by making use of radioactive or fluorescent labelled DNA/RNA probes complementary to the required sequence. However in paucibacillary states like tubercular meningitis and pleural effusion the number of bacilli is too low to be picked up by this technology. This technique is commercially available for the identification of M. tuberculosis complex, M. avium, M. intracellulare, M. kansasii and M. gordonae. Nucleic acid probes form a useful adjunct to BACTEC cultures for confirmatory identification.

3. Nucleic acid amplification
a. Polymerase chain reaction (PCR): PCR is an in vitro method for amplifying specific DNA sequence. Starting with extremely minute amounts of a particular nucleic acid sequence from any source, PCR enzymatically generates millions or billions of exact copies thereby making genetic analysis of tiny samples a relatively simple process. Studies in children have found PCR on respiratory samples including gastric lavage to have a sensitivity of 40-60% compared to clinical diagnosis. Nested PCR certainly enhances the sensitivity of PCR.

b. Transcription mediated amplification (TMA): TMA uses a species specific sequence of ribosomal RNA (rRNA) as the target for reverse transcriptase. The advantage of this technology being that the dead cells have no transcription machinery hence only viable cells are picked up and amplified.

DNA amplification technology can amplify minute quantities of DNA to levels that can be readily seen following routine agarose gel electrophoresis. But amplification can amplify even minute quantities of contaminating DNA. False positive results are the major concern. Also the presence of an organism in a clinical specimen does not necessarily indicate disease. Various target antigens have been used IS6110, MPB64, 16S rRNA gene 65kd etc.

Thus in conclusion the diagnosis of tuberculosis is not easy. Culture remains the gold standard and every attempt should be made to isolate the organism. In tubercular meningitis and pleural effusions adenosine deaminase is a useful adjunctive test. Serological tests do not offer an accurate sensitivity and should not be pursued. Lastly, molecular techniques certainly hold promise in diagnosis, but lack sensitivity in paucibacillary states.
Dr Camilla Rodrigues  
Consultant Microbiologist & Chairperson Infection Control Committee, MD – Microbiology.

EXPERTISE & EXPERIENCE:

Dr. Rodrigues set up the department of Immuno Serology and upgraded the Microbiology section of Lab Medicine to a state of the art lab with subsections of Bacteriology, Mycobacteriology, Mycology and Molecular infectious disease at Hinduja Hospital. Under her leadership and guidance, the section of Infectious Disease was established and obtained recognition for Infectious Diseases for a post doctoral fellowship by the National Board Examinations, New Delhi.

She has undertaken International collaborative projects with FIND (Foundation of Innovative New Diagnostics), Geneva, Fondation Merieux, France, University of Oxford, UK, Translational Research Unit Infectious Disease, Italy, Imperial College, UK etc. Additionally, in India she has conducted multicentric trials with DBT – Department of Science and Technology, Government of India and ICMR. She has been the microbiology coordinator of the yearly one year Infectious Diseases fellowship program at Hinduja Hospital which has been operational since 2004.

Recognized teacher for both M.Sc & PhD by Research in Applied Biology, Univ of Mumbai.
Recognised teacher for DNB Microbiology by the National Board Examinations. New Delhi
Her areas of interest include rapid diagnosis of infectious disease, antibiotic resistance and MDR/XDR tuberculosis.
AWARDS AND ACHIEVEMENTS

She has authored 132 publications in international and national journals and book, and has to date conducted 59 research projects as the chief or co-investigator with tuberculosis being a focused area of research. Under her guidance, a molecular test to identify Multi Drug Resistance (MDR) in Mycobacterium tuberculosis was developed for which a patent has been filed by the hospital.

She has received several awards & prizes - the most recent being the SC Agarwal award for outstanding contributions in the field of Medical Microbiology in 2009. She has given over 375 presentations, including orations, invited lectures as faculty in India and abroad.
The 7th Infectious Disease Certificate Course hosted by Hinduja Hospital in association with Henry Ford Health System, Detroit MI, USA will be held from 22nd August to 29th August 2010.

This year the course is structured from a basic level to a more advanced one, all within the span of 8 days. Topics with major impact will be emphasized including community and nosocomial infections like Tuberculosis, HIV, CNS infections, Skin & soft tissue infections, etc.

The course co-ordinators are Dr. F. D. Dastur Dr. Rajeev Soman, Dr. Camila Rodrigues and Dr. Anjali Shetty.

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Role of the Histopathology Laboratory in the diagnosis of Medical Renal Disease

The complex structure and function of the kidney renders it vulnerable to a wide variety of injurious stimuli that can affect the glomeruli, tubulo-interstitial compartment or the renal vasculature. The biopsy is a valuable diagnostic tool that gives information about the actual changes in the kidney. A meaningful interpretation of kidney biopsies is a clinicopathologic exercise. The aim of this article is to introduce some clinically relevant pathologic aspects.

The Pathologic Diagnosis of Glomerular Disease

The glomerular alterations following injury include: Capillary wall thickening, various types of proliferative changes, crescents, influx of neutrophils in the glomerulus, necrosis of tuft, capillary thrombi, glomerular deposits, glomerular sclerosis and several others.

Glomerular diseases may pose a diagnostic challenge as: 1) Some diseases may produce no alterations while in others major diagnostic features may be subtle. 2) Several diseases may show similar glomerular alterations and manifest with similar clinical syndromes. 3) A single disease may show a baffling variety of changes e.g. The spectrum of glomerular changes in IgA nephropathy and lupus nephritis ranges from normal to those with florid proliferative and sclerotic changes. The following queries and answers illustrate these situations.

Are normal glomeruli on light microscopy only a feature of minimal change disease (MCD)?
Apart from MCD, early membranous glomerulonephritis (MGN) and amyloidosis, mild examples of IgA nephropathy and lupus nephritis, Alport’s syndrome, storage disorders and even FSGS, may show no significant glomerular changes.

Are glomerular neutrophils seen only in post-infectious GN?
Activation of complement cascade with glomerular deposits of C3 is the cause for neutrophil influx in post-infectious glomerulonephritis and also some cases of class III and IV lupus nephritis & membranoproliferative GN. Neutrophils are also seen as response to glomerular endothelial damage as occurs in Hemolytic uremic syndrome (HUS).
Are terms such as membranoproliferative GN (MPGN), mesangioproliferative GN, membranous GN (MGN), crescentic GN final diagnostic entities? These are morphologic entities that describe the pattern of glomerular injury. Each of these may be secondary to a variety of actual disease processes. For example, MPGN and MGN may be idiopathic or secondary to lupus, HBV or HCV infections. MPGN pattern may also be observed in fibrillary and immunotactoid glomerulopathies and on occasion also in HUS. Mesangial proliferation may be encountered in lupus nephritis, resolving post-infectious GN or IgA nephropathy. Crescents may also be seen in a variety of diseases.

What is the role of immunofluorescence (IF) and electron microscopy (EM) in the diagnosis of glomerular disease? How should the biopsy be sent to the laboratory for these investigations? If examination helps in refining the diagnosis of glomerular diseases and in some situations is critical for achieving the diagnosis. For example early membranous GN may be diagnosed as minimal change lesion on light microscopy but IF may show the staining pattern typical of MGN. IF is essential for diagnosis of IgA nephropathy. Light chain disease may be diagnosed as MPGN unless IF examination using antibodies to kappa and lambda light chains are used. The classification of crescentic GN into pauci-immune, immune-complex and anti-GBM types is based entirely on IF examination. Correct techniques, familiarity with the patterns of staining and correlation with the light microscopy findings are essential for the proper interpretation of IF results. If biopsy for IF is being sent from another centre to our laboratory we provide vials of transport medium in which fresh biopsy tissue can remain for at least two days. Another method is to wrap the fresh tissue in aluminium foil and place it on an ice-pack or in a thermos packed with ice-cubes.

EM is essential for diagnosis of thin basement disease, Alport's syndrome and immunotactoid and fibrillary glomerulopathies. In most other instances it only serves as an useful adjunct to light microscopy. For EM, the tissue needs to be sent in 3% glutaraldehyde.

In summary it needs to be stressed that, accurate diagnosis of glomerular disease can be obtained only by correlation with the clinical setting.
Both primary and secondary TIN result in similar changes and hence in most instances decision regarding the etiology can be made only by co-relation with the clinical history.

The Pathologic Diagnosis of Tubulointerstitial Disease
The tubulointerstitial compartment is vulnerable to ischemia, toxins, infection, effects of drugs, radiation etc.and these result in primary TIN. It must also be remembered that tubulointerstitial damage may be secondary to glomerular and vascular disease.

Both primary and secondary TIN result in similar changes and hence in most instances decision regarding the etiology can be made only by co-relation with the clinical history.

Some types of TIN demonstrate a distinctive pattern. Granulomatous TIN is one such example that may be due to tuberculosis, sarcoidosis or drugs or may represent a reaction to vasculitis. Cast nephropathy in multiple myeloma results in severe TIN that may be so florid as to overshadow the distinctive casts. The pathologist must look for features of cast nephropathy in biopsies from elderly patients with acute renal failure and may in fact be the first to suggest this diagnosis. Primary hyperoxalurias result in end stage calculous renal disease and result in distinctive TIN due to massive deposits of oxalate crystals. The pathologist should recognize this pattern because the disease invariably recurs following isolated renal transplant.

The Pathologic Diagnosis of Vascular Renal Disease
Renal vascular damage may be secondary to vasculitis or hypertension. The injury may be directed to the vascular endothelium in as in TMA or to the smooth muscle as in calcineurin inhibitor toxicity. In vascular rejection, the inflammatory cells are to be looked for in the sub-endothelial space. Changes in the renal vasculature may not be as easily apparent as the more eye-
catching glomerular alterations especially if vessels of smaller caliber are involved. However, recognition of the injury and its pattern is important as it helps determine the cause of the lesion.

The Value of the Renal Allograft Biopsy
The transplant kidney can be beleaguered by a variety of complications that may share similar clinical manifestations. Some examples include:
1. The clinical features of acute rejection may be mimicked by acute tubular necrosis and BK polyoma virus nephropathy.
2. Post-transplant proteinuria may be due to recurrence of the original disease, a de novo GN or chronic allograft nephropathy.
3. Slow deterioration of the graft function may be a feature of chronic rejection or calcineurin inhibitor toxicity.

The kidney biopsy necessary to resolve these diagnostic dilemmas. Furthermore, C4d staining on the biopsy is required for the diagnosis of antibody mediated rejection which requires specific treatment modality.

The importance of the kidney biopsy in the transplant setting can be gauged from the following statement in Heptinstall’s Pathology of The Kidney, “Biopsy findings change the clinical diagnosis in an average of 36% of patients and therapy in 59% with no obvious diminishing value in the last 20 years. Biopsy results change therapy in both early and late post-transplant periods with approximately equal frequency”.

In summary, it is my experience, that regular interaction between the nephrologist and pathologist is essential. A pathologist with orientation towards nephropathology can extract the maximum possible information from the biopsy. This facilitates generation of a high quality kidney biopsy report that provides not just a mere morphologic diagnosis but also prognostic information and guides the clinician in the decision towards a lesion specific therapy.
Achievements

DR. Chitra Madiwale
Consultant Histopathologist & Cytopathologist, MD – Pathology

Expertise And Experience:
Dr. Chitra Madiwale has over 22 years of experience and her special areas of interest are nephropathology including renal transplant biopsies and gynecologic pathology.

Awards And Achievements:
Dr. Chitra Madiwale has written numerous articles for International and National journals in varied topics. “Renal lesions in AIDS: A biopsy and autopsy study” by Madiwale C, Venkataseshan VS published in the Indian Journal of Pathology Microbiology and “Acute renal failure due to crescentic glomerulonephritis complicating leprosy” by Madiwale CV, Mittal BV, Dixit M, Acharya VN published in the Nephrology Dial Transplant are of significance.

She was awarded the Fellowship in Nephropathology with Dr Jacob Churg and Dr Surya Venkataseshan at the Mount Sinai Institute of Medical Sciences, New York, USA and has been the convener for quiz session at the Maharashtra Pathology chapter conference (MAPCON) for a period of 3 years. She has also co-ordinated departmental efforts, while at KEMH that helped procure award for surgical pathology slide seminar at the state level MAPCON conference.
Digital Pathology—Global Perspective

Path Xchange, a global online pathology community, conducted its first trans-Pacific digital case conference on March 17, 2010. The virtual case conference included two leading hospitals in Mumbai, India -- Hinduja Hospital and Lilavati Hospital with the University of Nebraska Medical Center in the United States (UNMC).

Dr. Subodh Lele of UNMC, Dr. Anita Bhaduri and Dr. R. B. Deshpande of Hinduja Hospital, Dr. Chandralekha Tampi of Lilavati Hospital, and Dr. Meenal Hastak of Lilavati Hospital led the 1.5 hour live discussion over ten cases. The cases were scanned using BioImagene’s iScan Coreo™ system and uploaded on PathXchange.

“Utilizing PathXchange’s global collaboration capabilities to conference with pathologists half way around the world in real time was a unique and very rewarding experience for me. The cases from India that I saw and shared with my colleagues here in the U.S. were fascinating,” shared Dr. Lele.

The virtual collaboration meeting was held the day before a major inter-hospital conference – the Senior Surgical Pathologist’s Meeting (SSPM). Eighteen of Mumbai’s major teaching and private hospitals participated in reviewing the same cases during the SSPM, where results from the digital case conference were discussed.

“Hosting the virtual case conference on PathXchange allowed us to facilitate the exchange of information and gave a new dimension to expert opinion from across the globe. The slides looked excellent on screen and the exchange of ideas added value to the interpretative skills,” recounted Dr. Bhaduri.

The cases discussed during this conference can be accessed under the Senior Surgical Pathologist’s Meeting Group on PathXchange.
Digital Pathology –
An uncertain revolution

It is a digital world out there, and no one can escape it. Surgical Pathology which is back of the beyond as far as commercial interests are concerned, is now being targeted by Digital technology, and the life and practice of Surgical Pathologists is about to change for ever, at least that is what the technophiles promoting the new technology are saying.

Ten years ago when digital photography became a reality, surgical pathologists thought it was heaven; they could take as many pictures as they felt like, there was no cost or undue delay in processing the film and they could prepare a talk at a moment’s notice. What more could we ask!

Five years ago no one could even imagine digitizing a whole slide/section and turn it around as you pleased.

What is Digital Pathology, and how it will revolutionize the way we practice our discipline?

The current practice of surgical pathology is as follows

The tissues sent to the lab are fixed in formalin, processed in alcohol and xylene, embedded in paraffin to make paraffin blocks. These paraffin blocks are stuck in to microtomes to cut thin sections 5 to 7 micron thick. These are then stained with Haematoxyline and eosin (H & E) stains and examined under light microscopes. These paraffin blocks and H&E stained sections on glass slides are the working blocks of surgical pathologists. For good diagnosis, you need good and thin well stained sections, and a good microscope to examine the slides under. At least, that is how it is till now.

We need slides with stained sections on them to make a diagnosis. If we need a second opinion, say from another country, we have to send the slides by courier. More often than not, the glass slides get broken.

One quick way to get a second opinion would be to send digital images – many, many of them. This is not the ideal way, as the second Pathologist has to rely on your images and what you think is important.

Often Pathologists would like to see the whole section/slide themselves and decide what is important and what is not.

The new technology of digital Pathology is to have the whole...
The new technology of digital Pathology is to have the whole slide/section digitized by a scanner and offer it online. You do not need a microscope to view it; you need a computer. Digitized images can be sent by internet anywhere within a few minutes; and the Pathologist at the receiving end can sit at the computer and see the whole slide. He can move it as he likes; and see the images at different magnifications etc.

This new technology is going to revolutionize the practice of Surgical Pathology soon. There will be no glass slides on Pathologists' table. There will be only images on his computer sent from the Lab. There will be no need for microscopes; Pathologists will see the "slides" on computers. This means they need not go their office and to the microscope to report on the cases. They, like Radiologists, can report from anywhere, from their residences or from their campsites while fishing. This is all quite exciting really.

What are the advantages?
The main advantage is easy mobility. Second is the ease of storage of the slides and blocks. As of now, large space is occupied by the surgical Pathology departments for archiving the slides and blocks kept as patient records.

Another major advantage is the ease with which the images of the cases can be sent all over the world at the click of a button, and get a second opinion from anywhere in the world within a matter of minutes. Presently, the slides have to sent by courier. That takes days and there are Government regulations involved in sending biological materials. All these can be dispensed with the digital images.

There is now a limit on how many slides can be prepared from paraffin blocks especially from small biopsy tissue bits. This becomes a hindrance in supplying slides to students for learning purposes. With digital images, we can replicate without any limit. A student can carry in his bag thousands of images of hundreds of cases. All the slides of several hospitals can be carried in a pendrive; and the student can see these at his leisure any time; any where.

What are the disadvantages?
Old time pathologists who dominate the scene, (like the present writer), will certainly resist the change. Most of us are used to seeing the slides under the microscope. There is no way the great experts can be coaxed (at least at present) to report on computers! For them it would be
sacrilege! For this new technology to take root, it will take a few years. The new breed of pathologists who get trained on computers from day one will be comfortable with it.

One major disadvantage as, I see it, is that slides have to be prepared as we do now to digitize the images; there is no escaping from that. This new technology comes in only after the slides are prepared (at least one set), which can be digitized. So where is the real advantage? There will still be need for experienced technicians, there will still be need for all the equipment and reagents used for preparing the slides. So the time taken also will not be reduced. The only thing is it will add the cost of computer technology to what is now a low cost endeavor!

Major corporates involved in developing this new technology, however, are hopeful it will revolutionize the practice of Surgical Pathology, and that they will make huge profits! I doubt this will happen. We shall have to wait and see.

Achievements

Dr. Ramesh B. Deshpande,
Consultant Surgical Pathologist, MD – Pathology

Expertise And Experience
Dr. Ramesh B. Deshpande has more than 35 years of experience with special areas of interest being neuropathology, gastro-intestinal pathology, bone and soft tissue tumors, lymph node pathology, genito-urinary pathology. Has been actively involved with undergraduate and postgraduate teaching in pathology while working as Associate Professor in pathology at Seth GS Medical College and KEM Hospital, Parel, Mumbai.

Awards And Achievements
He has conducted several seminars in his specialty and has published articles in peer reviewed journals.

Email ID: dr_rdeshpande@hindujahospital.com Contact No. 24451515 ext 7159 / 7157
The College of American Pathologist conducted a seminar on CAP Laboratory Accreditation Program on 27th March 2010 at Hinduja Hospital. This was the 3rd time the seminar was held at the Hinduja Hospital. There were 102 registered delegates, 43 from Hinduja Hospital and 59 from 6 labs and other 5 hospitals of Mumbai. The introduction and overview was given by Dr. Bharati Jhaveri, the CAP State Commissioner for Illinois. The other speakers were Marcia Geotsalitis (Personnel & Safety Issues), M. Richard Scanlan (Proficiency Testing), Kailash B. Sharma (Preparing a Quality Management Program). This was followed by a lively interactive Q & A session and lunch. Some of the major participating hospitals were Jaslok Hospital, Bombay Hospital, Breach Candy Hospital, Holy Family Hospital & Seven Hills Hospital. The labs participating were Reliance Life Sciences Centre, Phadke Labs, Super Religare Labs, Metropolis Lab, Quintiles Lab & Medpace Labs. The seminar was appreciated greatly by all participants.
It is with great sorrow and deep sense of regret that we inform you of the sad demise of our dear Dr Ajita Mehta MD,DPB who passed away on 6th February 2010 in Mumbai. Known all over the country for her indomitable spirit, dynamism and warmth, Dr Mehta was born on the 27th June 1942. She graduated from Nagpur Medical College in 1966, and completed her DPB (1973) and MD (Pathology & Bacteriology) (1974) from the Seth G.S. Medical College in Mumbai.

She was an educator par excellence and a well loved teacher at the Seth G.S. Medical College where she established excellent standards for diagnostic microbiology. She was a visionary microbiologist who understood the importance of daily interactions with the clinicians for optimizing the care of patients with infectious diseases. She was one of the first microbiologists in the country who actually took microbiology from the bench to the bedside.

Extremely compassionate, popular and generous, she was known as the doctor with the golden heart. She was passionate about her work and would go out of her way to encourage and help others to establish good microbiology practices. She was greatly loved by all her staff, students and colleagues for being the really special person that she was. Courageous, fearless and outspoken, she was not deterred from attaining her goals.

Dr Mehta was indeed a pioneer particularly in 2 areas of microbiology: namely that of anaerobic bacteriology and Hospital Infection Control. She alone was largely responsible for creating awareness and setting up Hospital Infection Control Programmes at various centres all over India.

At the Hinduja hospital, she spearheaded Infection Control activities from surveillance and waste disposal to handrub and needlestick injury prevention with a zeal that was unmatched. She also started the Infection Prevention Week from 1992 onwards in an effort to create awareness amongst all levels of health care workers including doctors, nurses, technologists, and attendants.
Appointments
Dr Mehta served as a Lecturer in Pathology and Microbiology at the Seth G.S. Medical College, Mumbai from January 1969 to August, 1974 and an Assistant Professor / Reader at the same institute from September 1974 to June 1989. She then joined the Hinduja Hospital on 1st July 1989 where she served as Consultant Microbiologist and Chairperson of the Infection Control Committee till her retirement in June 2008. Subsequently she took up the Chair of Infection Control 2008-2009 at Nanavati Hospital in Mumbai.

Teaching experience
Dr Ajita Mehta taught undergraduate students of 2nd MBBS for 20 years from 1969-1989 and was a post graduate teacher in Microbiology from 1978-1989. At the induja hospital, she was a recognised teacher for M Sc and Ph D in Applied Biology in addition to being a guide for DNB Microbiology.

Associations
At a national level she was the President of Hospital Infection Society, India (HIS-I) from 2001 till 2006 which she was instrumental in founding in 1992. She also was the Chairperson of the Hospital Infection Society, Mumbai Forum and Vice President of the Infectious Disease Society India.

Farewell dear Ajita......
Flow cytometry is the measurement (metry) of properties of cells (cyto) as they flow in a single fluid stream (flow), past a stationery set of detectors. It is capable of rapid quantitative, multi-parameter analysis of heterogeneous cell populations on a cell-by-cell basis (single cell analysis) using laser based technology. Characteristics that can be measured include cell size, cytoplasmic complexity, DNA or RNA content, and a wide range of membrane-bound and intracellular proteins. The FC generated pattern can then be interpreted for the definitive rapid diagnosis of non-malignant and malignant disorders. The major benefit of this technology is its capacity to rapidly measure and record multiple characteristics of a larger number of cells at rates that exceed 5,000 cells per second, so that even low frequency populations (eg small number of abnormal/malignant cells) can be quantitated with a high degree of statistical accuracy.

Flow cytometry is used for immunophenotyping of a variety of specimens, including whole blood, bone marrow, serous cavity fluids, cerebrospinal fluid, urine, and solid tissues. This review will describe the basic principles of flow cytometry and provide an overview of some applications in hematology. Some of these are offered as tests for patient care.

**General Principles**

Flow cytometry measures optical and fluorescence characteristics of single cells or any other particle, including nuclei, microorganisms, chromosome preparations, and...
Flow cytometry simultaneously measures and then analyzes multiple physical characteristics of single particles, usually cells, as they flow in a fluid stream through a beam of light.

Latex beads. Physical properties, such as size represented by forward angle light scatter (FSC) and internal complexity represented by right-angle scatter (SSC) can resolve certain cell populations. Additionally, antibodies conjugated to fluorescent dyes can bind specific proteins on cell membranes or inside cells. When labeled cells are passed by a light source, the fluorescent molecules are excited to a higher energy state. Upon returning to their resting states, the fluorochromes emit light energy at higher wavelengths. The use of multiple fluorochromes, each with similar excitation wavelengths and different emission wavelengths (or “colors”), allows several cell properties to be measured simultaneously.

- **The fluidics system** transports particles in a stream to the laser beam for interrogation.
- **The optics system** consists of lasers to illuminate the particles in the sample stream and optical filters to direct the resulting light signals to the appropriate detectors.
- **The electronics system** converts the detected light signals into electronic signals that can be processed by the computer. For some instruments equipped with a sorting feature, the electronics system is also capable of initiating sorting decisions to charge and deflect particles. In this way, particles of interest can be collected in a pure state.

**Applications in Hematology**

(i) **Analysis of CD4 and CD8 lymphocyte subsets**

This assay is performed for immunologic monitoring of HIV positive patients. HIV infects helper/inducer T lymphocytes via the CD4 antigen. Infected lymphocytes may be lysed when new virions are released or may be removed by the cellular immune system. As HIV disease progresses, CD4-positive T lymphocytes decrease in total number. The absolute CD4 count provides a powerful laboratory measurement for predicting,
Flow cytometry is very effective in distinguishing myeloid and lymphoid lineages in acute leukemias and minimally differentiated leukemias.

Flow cytometry is very effective in distinguishing myeloid and lymphoid lineages in acute leukemias and minimally differentiated leukemias.

staging, and monitoring disease progression and response to treatment in HIV-infected individuals.

Quantitative viral load testing is a complementary test for clinical monitoring of disease and is correlated inversely to CD4 counts. However, CD4 counts directly assess the patient’s immune status and not just the amount of virus. It is likely that both CD4 T-cell enumeration and HIV viral load will continue to be used for diagnosis, prognosis, and therapeutic management of HIV-infected persons.

We offer both CD4 enumeration and viral load studies for management of HIV positive patients.

(ii) Immunophenotyping of hematolymphoid malignancies

Perhaps the best example of simultaneous analysis of multiple characteristics by flow cytometry involves the immunophenotyping (immunological characterization) of leukemias and lymphomas. Diagnostic interpretations depend on a combination of antigen patterns and fluorescence intensity. Flow cytometry is very effective in distinguishing myeloid and lymphoid lineages in acute leukemias and minimally differentiated leukemias.
The goal in B-lineage ALL is to determine the stage at which the leukemic cell has been arrested in the ontogeny of B-cell maturation. Differentiated leukemias.

An extensive panel of antibodies used upfront helps characterization of a wide range of immature and mature hematopoietic cells in the sample and increases the sensitivity of the test when malignant cells are only in a minority. We use this approach to diagnose both acute leukemias as well as chronic lymphoproliferative disorders (CLPDs).

(i) Distinction of acute myeloid leukemia (AML) from acute lymphoblastic leukemia (ALL)

One of the most important applications is to define the cell of origin of acute leukemia. Lineage derivation can be precisely determined by the analysis of functionally important cytoplasmic antigens such as myeloperoxidase for myeloid cells, CD79 and CD22 for B-cell lineage and CD3 for T-cell lineage. Further membrane antigens such as CD33 and CD13 for myeloid, CD19 for B-cell and CD7 for T-cell lineage are also diagnostically helpful, but they are not always as precise as the detection by cytoplasmic markers. The additional analysis of CDw65 and CD117 may increase the sensitivity for the detection of myeloid cells.

Taken together, the detection of myeloperoxidase plus CD13/CD33 (myeloid), CD79 plus CD19 (B-cells) and CD3 plus CD7 (T-cells) respectively identifies >98% of acute leukemias as myeloid, B lymphoid or T lymphoid thus allocating 1.5-2% into the acute undifferentiated category.

(ii) Classification of AML subtypes

Immunophenotyping also contributes to the further characterization of AML subtypes, especially the M0 and M7 subtype where it is mandatory for diagnosis. While the French American British (FAB) definition of M1 to M6 is still based mainly on morphology and cytochemistry, recently it has been observed that biologically defined subsets of AML such as the t(8;21) karyotype is associated with FAB M2 morphology, the inv(16) karyotype with FAB M4 morphology and APL with t(15;17). In addition, these entities also express a characteristic immunophenotype.

(iii) Classification of B-lineage ALL subtypes

The goal in B-lineage ALL is to
We have been among the first labs to perform immunophenotyping of acute leukemias and CLPDs on the flow cytometer not only in the city of Mumbai but all across the country.

(iv) Classification of T-ALL subtypes
The goal is to discriminate between T-ALL and mature T-cell malignancies. Between 15-20% of ALL cases arise from the T lineage. These cells express CD2, CD3, CD5, CD7. CD10 may also be present (this signifies a better prognosis) but CD 19 and CD 20 are not. Furthermore the determination of CD4 and CD8 and their co expression may be helpful in the analysis of T cell (im) maturity.

(v) Chronic lymphoproliferative disorders (CLPD)
For the analysis of chronic lymphocytic leukemia (CLL) and B-lineage non-Hodgkin lymphoma, monoclonal antibodies or heterologous antisera against surface immunoglobulin light chains (kappa and lambda) in combination with CD19 are used for the analysis of a clonal expansion of B-cells. A panel of antibodies is used to differentiate the various CLPDs such as CLL, mantle cell lymphoma, hairy cell leukemia, follicular lymphoma, splenic lymphoma and marginal zone lymphomas. Expression of specific molecules at time of diagnosis such as CD38 and ZAP70 in CLL is associated with poor long term survival. The cellular analysis in chronic leukemias of T-lymphocyte lineage is based on the expression of pan T-cell associated antigens such as CD3, CD2, DC7 and CD5 together with antigens such as CD4, CD8, CD56 and CD57 showing associations to subtypes of chronic leukemias of T-lymphocyte lineage. Further information can be gained from the aberrant or missing expression of T-cell antigens because peripheral
The flow cytometer has been used for the analysis of platelet structure and function in the research laboratory.

T cell lymphomas variably show aberrations from the typical features of peripheral T cells. These diagnostically important changes, unique to each case, may include CD3 expression in aberrantly low density, lack of CD2 or CD7, or negativity or positivity for both CD4 and CD8 expression.

Types of specimen
Immunophenotyping of nucleated hematopoietic cells can be performed using material taken from a variety of body compartments. Bone marrow is the preferred material for immunophenotyping in acute leukemia. Other materials useful for immunophenotyping of hematopoietic cells include, peripheral blood, malignant effusions, e.g. ascitic or pleural fluid and solid tissue, e.g. lymph nodes, after preparation of single cell suspensions.

Specimen collection
The test is performed on heparinized or EDTA samples. Specimens should be transported to the flow cytometry laboratory as soon as possible. Information about age, sex, presumptive diagnosis, differential blood count, current therapy as well as the status of lymph nodes and spleen of the patient should be provided on the test requisition form. In addition, smears should be prepared for morphological and cytochemical analysis (when necessary). If bone marrow is collected, white blood cell differential counts should be performed simultaneously from bone marrow and peripheral blood in order to check the degree of blood contamination in the aspirate and the number of blasts in the blood.

We have been among the first labs to perform immunophenotyping of acute leukemias and CLPDs on the flow cytometer not only in the city of Mumbai but all across the country.

*HLA B27 analysis*
A flow cytometric assay for lymphocyte HLA-B27 expression using two-color direct immunofluorescence is available. For the assay, whole blood is mixed with a monoclonal anti-B27 conjugated to fluorescein-isothiocyanate (FITC) and anti-CD3 conjugated to phycoerythrin (PE). The samples are analyzed for anti-B27 staining intensity.

The HLA-B27 test is used as one of the parameters in a constellation of signs, symptoms, and lab tests to support or rule out the diagnosis of certain autoimmune disorders, such as Ankylosing Spondylysis and Reiters syndrome. The HLA-B27 test may be ordered as part of a group of tests.
We are the only lab clinical performing platelet antigen assay for diagnosis of the hereditary platelet function disorder, Glanzmann thrombasthenia.

- **Platelet Function Analysis**
  The flow cytometer has been used for the analysis of platelet structure and function in the research laboratory. Although the small physical size and biovariability of the platelet creates inherent difficulties for flow cytometric analysis, several clinical assays are performed by specialized flow cytometry laboratories. These assays have been classified by Bode and Hickerson to include:
  1) platelet surface receptor quantitation and distribution for the diagnosis of **congenital platelet function disorders**
  2) platelet-associated IgG quantitation for the diagnosis of **immune thrombocytopenias**
  and for platelet cross-matching in transfusion
  3) reticulated platelet assay to detect "stress" platelets
  4) fibrinogen receptor occupancy studies for monitoring the clinical efficacy of **platelet-directed anticoagulation in thrombosis**,
  and 5) the detection of activated platelet surface markers, cytoplasmic calcium ion measurements, and platelet microparticles for the assessment of **hypercoagulable states**

We are the only clinical lab performing platelet antigen assay for diagnosis of the hereditary platelet function disorder, Glanzmann Thrombasthenia (G T).

**Other clinical applications of flow cytometry are:**

- **Detection of minimal residual disease (MRD).** The persistence of malignant cells in the bone marrow or other tissues in patients of hematological malignancies after remission cannot be detected by conventional microscopy because these cells are present in very small numbers and it is believed that these cells are responsible for relapse in many patients. At present we have a research project for MRD which will shortly become available for patient testing.

- **Analysis of DNA ploidy, the Cell Cycle and Cell Death:**
  This is most often performed in patients with node-negative breast cancer where clinical correlation is strongest.

- **Reticulocyte enumeration** by flow cytometry is much more accurate and precise than manual counting. The FC gives additional information such as the maturation index (RMI) and the immature reticulocyte fraction (IRF) which is valuable in clinical diagnosis and
Flow cytometric estimation of CD41, CD61 for diagnosis of GT

- Monitoring of anemia and other diseases.
  - **Detection of feto-maternal hemorrhage** is more accurate and sensitive compared to the manual method of Kleihauer and Betke.
  - **Paroxysmal Nocturnal Hemoglobinuria (PNH)** is an acquired clonal stem cell disorder that leads to intravascular hemolysis. Conventional lab tests performed were Ham’s and Sucrose lysis. Flow cytometry is the most sensitive and accurate method of diagnosing PNH. It is characterized by antibodies to CD55 and CD59 on red cells and leucocytes which can be detected by flow cytometry.
  - **Applications in organ transplantation** include pre transplant cross matching, HLA antibody screening and post transplant antibody monitoring.
Detection of feto-maternal hemorrhage is more accurate and sensitive compared to the manual method of Kleihauer and Betke.

- **Enumeration of CD34 positive cells in bone marrow or peripheral blood transplantation** correlates with engraftment success and length of hematopoietic recovery. The applications of FC in bone marrow transplantation include graft rejection, graft versus host disease and graft versus leukemia effect.

**Future Expectations**
The flow cytometer is the only instrument that provides multiparametric analysis at the single cell level. New fluorochromes, including UV-excited, complex of dyes (tandem dyes), and nanocrystals are under development, as well as a new generation of modular flow cytometers using small, solid state lasers, robotics, and advanced, innovative bioinformatics software, like the laser scanning cytometer (LSC), a microscope slide-based technology capable which is proving particularly useful for the analysis of fine needle aspirate and body fluid specimens. Some of the research applications which are expected to cross over into the clinical laboratory are hematopoietic stem cell differentiation, multi-drug resistance studies (MDR) in cancer and platelet activation and analysis in coronary artery disease.
**Expertise And Experience**

Dr. Khodaiji has over 30 years of post M.D experience in the field of laboratory hematology. She has received extensive training abroad and in India in diagnostic hematopathology and ancillary laboratory assays such as flow cytometric immunophenotyping and cytogenetics. Her special areas of interest are:

**Benign Hematology**
- Coagulation and platelet disorders and thrombophilic states.
- Anemia diagnosis and management with special focus on developing early indicators of iron deficiency anemia.

**Hemato-oncology**
- Diagnosis of leukemia and lymphoma with special focus on newer investigative modalities such as flow cytometry and molecular markers.

**Flow Cytometry**
- Conventional and newer applications

**Awards And Achievements**

Dr. Khodaiji has organized several CMEs in hematology. She has been invited as a guest lecturer for national conferences and CMEs. She was elected secretary of the Mumbai Hematology Group. At Hinduja Hospital she has been the principal investigator of numerous research projects. She has won many awards as well as the prestigious BGRC oration at the 32nd annual conference of the Mumbai Hematology Group in Mumbai (2009). Dr. Khodaiji has published numerous book chapters and articles in both international and national journals.

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Email : dr_skhodaiji@hindujahospital.com, Contact No. 24451515 ext 7144
Laboratory Diagnosis of Fungal Infection

The incidence of invasive fungal infections has increased in the last two decades. Diagnosis of infections such as cryptococcal meningitis is straightforward but diagnosing invasive aspergillosis can be problematic. No matter how experienced the mycologist, isolation and identification of fungi from clinical specimens are not likely to be accomplished unless the specimen is properly collected and sent immediately to the laboratory.

Specimen Collection for skin, hair and nail infections
Samples NOT needed for
- Uncomplicated athlete’s foot
- Mild infections of the groin
- Mild skin ringworm

Samples for fungi needed
- When oral treatment is being considered – scalp ringworm or nail disease
- In severe skin fungal infections
- Skin infections refractory to initial treatment
- When the diagnosis is uncertain

Sample Collection For Fungal Investigation
- Swabs are of no value for dermatophytes
- Clean the area with 70% alcohol to get rid of creams, lotions etc.
- Keep any samples at ROOM temperature, do NOT refrigerate.

Skin:
- Scrape skin from the advancing edge of lesion. Use a blunt scalpel blade.

Nail samples:
- In superficial infections scrape surface of infected nail plate with scalpel blade
- Most viable fungi are usually found in most proximal part of diseased nail
- Clippings should include full thickness of the nail and extend as far back from nail tip as possible

Hair samples:
- Pluck hair from affected areas and take skin scrapings from associated scalp lesions (hair alone is insufficient)

Microscopy
Microscopy provides a better yield than that for culture alone. It is also a rapid test and the results are available in a few hours. Microscopy helps differentiate between infection caused by a septate or aseptate mould. At Hinduja we use a fluorescent stain (Calcofluor) to stain the cell wall chitin of fungi.
Diagnosis of infections such as cryptococcal meningitis is straightforward but diagnosing invasive aspergillosis can be problematic. This increases the rapidity of performing microscopy and improves sensitivity.

Mucorales are particularly susceptible to chilling in the refrigerator and tissue homogenization. Hence quite often they fail to grow and microscopy is the only means of establishing a diagnosis.

**Histopathology**
All tissues from immuno-compromised (including patients on steroids) with suspected fungal infection should be stained with fungal stains. It is important that the clinician mentions on the form that the patient is immunocompromised and requests fungal stains because routine haematoxylin and eosin stains are unlikely to stain fungi.

**Blood cultures**
Blood cultures for fungi should be sent in BACTEC Myco/Flytic BACTEC bottles. This is a liquid medium developed to increase isolation of fungi and mycobacteria. It contains Middlebrook 749 and Brain Heart Infusion Broth. Ferric ammonium citrate is added as an iron source for fungi. Saponin is added to lyse the blood.
It is important for laboratories to culture samples on specialized fungal media such as Sabouraud dextrose agar provide a higher yield of fungi such as Aspergillus.

The sensitivity of blood cultures to detect candidaemia is only 50%. Aspergillus is cultured extremely rarely from blood cultures. It is recommended that at least 20 ml of blood should be cultured to increase the yield.

**Fungal cultures**

It is important for laboratories to culture samples on specialized fungal media such as Sabouraud dextrose agar provide a higher yield of fungi such as Aspergillus. Prolonged incubation of cultures for two to three weeks at 30°C is essential to increase the yield of fungus.

**Identification of fungi**

All fungi isolated from sterile sites should be identified to species level. The reason being that the susceptibility to antifungals varies with the type of fungus. Candida krusei is intrinsically resistant to fluconazole. Candida glabrata is susceptible dose dependent to fluconazole. Candida parapsilosis has higher MICs to caspofungin.

We use chromogenic agar to identify the common species of candida. This agar facilitates rapid isolation of yeasts from mixed cultures and allows differentiation of Candida species namely Candida albicans, Candida krusei, Candida tropicalis, and Candida glabrata on the basis of colour and colony morphology. Results are obtained within 48 hours.

We also use ID 32 C which is a standardized system for the identification of yeasts. This test uses 32 miniaturized assimilation tests and a database. It takes 48 hours to give a result.

**Susceptibility testing**

With the increase in azole resistance in Candida albicans and non-albicans Candida, it is important to perform susceptibility tests for the various antifungals. We use ATB Fungus 3 which enables the determination of the susceptibility of Candida and Cryptococcus neoformans to antifungal agents in a semi-solid medium. The result obtained gives a MIC for Amphotericin B, Fluconazole, Itraconazole and Voriconazole. It classifies organisms as being sensitive, intermediate or resistant to flucytosine.

**Antigen testing**

The serologic tests for detection of cryptococcal polysaccharide antigen in serum and CSF are extremely accurate for the diagnosis of invasive cryptococcal disease. Latex agglutination tests are greater than 90% sensitive and specific. This test supercedes India ink staining for CSF as the
With the increase in azole resistance in Candida albicans and non-albicans Candida, it is important to perform susceptibility tests for the various antifungals. 

sensitivity for India ink is 50% for non-AIDS and 80% for AIDS patients with cryptococcal meningitis. The antigen test has two advantages; better sensitivity and the fact that serum may be used to diagnose the condition.

It is vital for a clinician to know the tests available to diagnose this group of infections. Using the laboratory effectively would assist the clinician to accurately diagnose fungal infections.

Achievements

Dr. Anjali Shetty
Consultant Microbiology, MRCP, FRCPath – Pathology

Expertise And Experience:
Dr. Anjali Shetty has experience of over 11 years having worked both abroad and in India. Her special areas of interest are clinical bacteriology and infection control.

Awards And Achievements:
Dr. Anjali Shetty has numerous publications to her credit in both international and national journals and recently the “Outbreak Of Listeria Monocytogenes In An Oncology Unit Associated With Sandwiches Consumed In Hospital” along with Davies E, Mclaughlin J, Grant C, Ribeiro CD was published in the Journal of Hospital Infection.

She has been awarded the Travel Bursary Award by the Hospital Infection Society to lecture in South Africa on Infection Control and for the best oral presentation at BIS in May 2005 for “A Case Of Colonic Stricture In A Neonate” along with Barnes RA.
Role of the Pathologist in the management of breast diseases

Surgical oncologic practice in breast cancer thrives on robust pathology support. With approximately 75,000 new cases of breast cancer occurring in India per year, breast cancer is the second most common malignancy with an AAR in Mumbai of about 30 cases per 100,000.

Breast cancer is a diverse group of diseases in terms of presentation, morphology and molecular profiling. Pathologists over the years have witnessed the trends and swings from radical surgery to conserving lumpectomies of the breast. Today, the trend is to excise less and conserve more. As a result pathologists are being forced to give maximum information on smaller tissues. Also, the techniques of special handling of the smaller excisions in an appropriate manner have evolved in many specialized centres. Therefore, an accurate description and adequate reporting has become mandatory.

The contents of a good pathology report describe not only the nature of the lesion but also the type, grade, vascular invasion, associated lesions of ADH/LCIS/DCIS/microinvasive carcinoma. Then the presence and number of nodal metastases and margin status. Hormone receptor evaluation (ER/PR status) is no more an optional

Fig: Micropapillary change in a columnar cell lesion H&Ex400)
A negative FNA report on a poorly cellular aspirate is actually an inconclusive report or inadequate report rather than a true negative. In all cases of positive cytological diagnoses, the triple test has to concur before embarking on radical procedures.

The Pathologist’s interpretation of tissue is imperative towards executing major excisions of the breast, starting from the primary diagnosis of benign versus malignant on an unequivocal cytology in an Fine Needle Aspirate (FNA) or on a core biopsy or excisional biopsy. The primary lab screening test for establishing malignancy is FNAC which has been very effective with accuracy rates of 95-99%. It is important to realize that only cellular aspirates are of diagnostic value. A negative FNA report on a poorly cellular aspirate is actually an inconclusive report or inadequate report rather than a true negative. In all cases of positive cytological diagnoses, the triple test has to concur before embarking on radical procedures.

The last decade, with the advent of neoadjuvant chemotherapy, has seen a new era of tissue core biopsies evolving. Also, since cytology evaluates cells that have been shed it is not possible to comment on whether the tumor is an in-situ carcinoma or an invasive carcinoma and hence is aptly labeled as ‘ductal carcinoma only’ on an FNA. Confidence levels in providing correct FNA reports should be in the range of 99-100% to enable the surgeon to directly carry out an excisional procedure without having to resort to a frozen section. Since the nineties, the frozen section is mainly used to establish the completeness of excision with evaluation of margin status in breast conservations or in sentinel node biopsies.

The sentinel node is another new technique that gained popularity by the late nineties in picking out microscopic metastatic disease for patients undergoing breast conservation who appeared clinically node negative. In experienced hands it has a negative predictive value of 95%. The management decisions in breast cancer hinge on pathologic interpretation of tissues as regards the predictors of risk of tumor recurrence e.g. tumor type and size, number of metastatic nodes, lymph vascular invasion, extensive DCIS component etc.

Modern day pathologists recognize a number of good prognostic types of special invasive tumors e.g. tubular, cribriform, papillary...
Infiltrating lobular carcinomas are difficult to pick up on radiology as they do not calcify and are nonpalpable very frequently. And mucinous as opposed to the bad prognostic types of basal, inflammatory, metaplastic and micropapillary types. Infiltrating lobular carcinomas are difficult to pick up on radiology as they do not calcify and are nonpalpable very frequently. These are often missed by pathologists as well since they show very subtle nuclear abnormalities on FNAs and yield paucicellular aspirates. The incidence of invasive lobular carcinomas has seen a rise following introduction of HRT and better detection with E-cadherin IHC.

Pleomorphic ILC is frequent in postmenopausal women and is the most common type in Locally advanced tumors (LABC). Micropapillary type shows clear spaces with an inverted papillary pattern and is an aggressive variant. It is frequent in young women in the Indian subcontinent and shows high angioinvasive potential with skin involvement. Gene microarrays have helped recognize the basal phenotype as a subset that are ER neg PR neg CerbB2 negative (triple negative) but EGFR and CK5/6 positive to categorise it in the basal or myoepithelial phenotype. This is easily remembered as the ‘triple negative phenotype’ of high grade breast cancer. The triple negative basal type of carcinoma and the micropapillary type are more common in India than elsewhere in the world. The metaplastic types include the spindled sarcomatous types and matrix producing carcinomas. These have the basal phenotype as well and uniformly show poor prognosis.

The Modified Bloom – Richardson scoring system is used to grade all types of invasive cancers and the Van Nuys Prognostic index for pure in-situ carcinomas. Metastases in the breast masquerading as primary malignancies are not unknown. The most common primaries to metastasize to the breast are the lung, stomach, ovary and prostate apart from hematolymphoid tumors. Rhabdomyosarcomas and renal cell carcinomas also show frequent metastases to the breast.

Non – Palpable breast lesions
The general acceptance of screening mammography and popularity of breast MRIs brought along their own problems of detection of several varieties of non-palpable lesions that were radiologically suspect for malignancy. Surgical Pathologists at the start of the century were therefore, having to cope with the nomenclature of these hitherto undescribed entities and having to familiarize themselves with their descriptions and prognostic significance of each.
The development of breast cancer is now believed to be a complex multistep process originating in terminal duct lobular units and progressing towards invasive cancer.

Communication, therefore, became imperative with the radiologists, oncosurgeons and the medical oncologists and thus developed the “team approach” to tailor-made therapies of these very early non-palpable lesions. The study of these lesions is tedious and time-consuming and needs dedicated pathologists to pick up abnormalities that have a bearing on the treatment approach. Most of these lesions show microcalcifications and spiculations that can mimic benign lesions like the Radial Scar. A high proportion of FEs and ADH as well as DCIS are noted in screen-detected breast lesions.

The development of breast cancer is now believed to be a complex multistep process originating in terminal duct lobular units and progressing towards invasive cancer. Previously, about 4 precursor lesions were known that separated normal and malignant epithelia in the breast, namely ADH, ALH, LCIS and DCIS. Then, there was a fifth group called CCLs that were clonal and neoplastic lesions and appeared closely associated in the development of low grade, special types of breast tumors. There are now six recognized entities of columnar cell lesions as CCC, CCH, CCH with architectural atypia, CCH with cytologic atypia, CCH with both cytologic and architectural atypia and CCC with cytologic atypia. The CCLs consistently harbor recurrent chromosomal abnormalities reinforcing the belief that they are neoplastic lesions leading to the development of low

Fig: Columnar cell change with hyperplasia but no atypia (H&E x400)
A high degree of only PR+ tumours in any laboratory needs a quality check on ER antigen retrieval methods.

The Myth of differing types of breast cancers in the West vs East

Adjuvant therapy of breast cancer is completely dependent on the pathologist’s interpretation and evaluation of hormone receptor status of tumors. The hormone receptor status can be evaluated on IHC staining of formalin fixed paraffin embedded sections of the primary tumor or its metastatic foci. There is automation available today for the staining procedure that has helped resolve a lot of the problems encountered with manual staining in the earlier protocols. The earlier data that suggested that Indian women have a different type of breast cancer as compared with the West i.e. an ER-PR+ phenotype or an ER-PR- phenotype was a myth that was shattered with improved staining techniques. Also, CerbB2 staining for detecting tumor types that would benefit from Herceptin therapy is pretty much routine now and forms a part of standard reporting format of any invasive breast cancer.

We have looked at our own current ER/PR receptor expression (HH) data as well as data generated from Tata Memorial Hospital (TMC) and have discovered that it is no different in expression of receptors in the Indian population from the Western World. About 50% tumours show overall receptor expression with 40% being in the ER+PR+ in Indian women and only PR+ in <5%. The tumour grades also show variation in ER/PR expression. A high degree of ‘only PR+ tumours’ in any laboratory needs a quality check on ER antigen retrieval methods. Shortcuts with only ER testing may not allow us to get the benefit of this “quality check” that is obvious when PR is also tested simultaneously. A consensus panel of National Institutes of Health, USA, recommended that any degree of ER staining in breast cancer should be considered as ER positive tumour and the patient a suitable candidate for endocrine therapy. So the cut off points have been lowered for reporting hormone receptor positivity. ASCO-CAP guidelines recommend using 5% positivity as cut-off.

Management strategies in breast cancer today are heavily dependant on the pathologic interpretation of tissues. Planning of adjuvant therapy and predicting risk of recurrence is dependent on the results in biopsies generated in the surgical pathology report. Thus there is an emerging significant role for the surgical pathologist in the breast working group of every hospital to achieve “standards of care” at par with the best in the world.
Dr (Mrs.) Anita S. Bhaduri
Consultant Histopathologist and Cytologist, MD - Pathology

EXPERTISE AND EXPERIENCE
Dr. Bhaduri has been practicing surgical pathology and cytopathology for close to thirty years in a teaching-cum-tertiary care hospital. Her areas of special interest are oncologic pathology with emphasis on breast and gynaec pathology and applications of immune-histochemical and molecular studies in diagnostic pathology. She has been the Guide for Post doctoral Certificate Course in Oncosurgical pathology (PDCC) conducted by the Indian College of Pathologists. Two successful candidates have passed out under her guidance since 2006.

AWARDS AND ACHIEVEMENTS
Dr. Bhaduri has the distinction of being awarded the British Council TCTP Award for advanced Surgical Pathology Training by the Royal Postgraduate Medical School London and University College of Medical Sciences, London, U.K and has been the member of the New York Academy Of Sciences since 2000.

She has also been the organizing secretary and conducted a number of workshops like the 11th Annual IAP-ID CME (Mumbai), A.P.Chapter Conf. of IAPM (Pune) and the most recent being the 6th Asia-Pacific International Academy of Pathology Congress (2009) held at Kochy.

Dr. Bhaduri has written several articles on various topics and has co-authored the “Guidelines in Breast Pathology Reporting” and recently the “Post-transplant lymphoproliferative disorder in GIT”. Dr. Bhaduri has been on the National Editorial Board of Indian Journal of Cytology & Indian Journal of Pathology & Microbiology.
1. Estimation of the bacteriologic efficacy of a peptide for Mycobacterium tuberculosis.
   Dr. Z.F. Udwadia

2. To study incidence of Latent iron deficiency anemia.
   Dr. S. Khodaiji

3. Evaluation of antifungal susceptibility testing of candida species by disk diffusion test by comparing it with MIC BMD according to CLSI guidelines. The sensitivity and specificity of disk diffusion will be calculated.
   Dr. Camilla Rodrigues

4. Early detection of Invasive fungal infections in Immunocompromised patients (Part II)
   Dr. Camilla Rodrigues

5. Prospective In-Vitro activity of Daptomycin against gram-positive cocci: a multicentric study in India
   Dr. Camilla Rodrigues
New Tests Introduced

Biochemistry
- Cystic fibrosis for 30 mutations
- P1NP

Microbiology / Serology
- Kala Azar
- H1N1
- Influenza A/B
- RLBH - rapid diagnosis of MDR TB

RIA
- Human Endothelin 1
- Placental Growth factor
- Vascular Endothelial growth factor
- Soluble VEGFR1/FLT-1
- Allergy package occupational
- Allergy package Addl foods
- Aquaporin 4
- Anti - Mullerian Hormone

Histopathology
- C4d staining by IHC in renal biopsies

For more details about our lab services, please contact
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Printed and Published:
by Marketing Department Hinduja Hospital, Veer Savarakar Marg, Mahim, Mumbai - 400016
at Synergy Creations for free and private circulation.
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